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Smooth Muscle Cell Abundance and Fibroblast Growth Factors in Coronary Lesions of Patients With Nonfatal Unstable Angina

A Clue to the Mechanism of Transformation From the Stable to the Unstable Clinical State

Moshe Y. Flugelman, MD; Renu Virmani, MD; Rosaly Correa, MD; Zu-Xi Yu, MD; Andrew Farb, MD; Martin B. Leon, MD; Amir Elami, MD; Ya-Min Fu, MD; Ward Casscells, MD; Stephen E. Epstein, MD

Background. **The mechanisms responsible for the transformation of stable angina to unstable angina, a major cause of morbidity and mortality, are commonly believed to be plaque rupture and thrombosis. We determined whether additional mechanisms are operative by analyzing the histopathology and immunohistopathology of coronary plaques retrieved by directional atherectomy of patients with unstable angina in whom no intraluminal thrombus was demonstrated by angiography.**

Methods and Results. **The histological findings of atherectomy specimens from 34 patients with unstable angina were compared with those of 24 patients with postangioplasty restenosis, whose lesions are known to be composed of smooth muscle cells (SMCs), and 10 patients with stable angina, whose lesions contain relatively few SMCs. We also studied the expression of acidic and basic fibroblast growth factors (aFGF and bFGF), whose role in the vascular response to injury has been established. Specimens from unstable angina resembled those from postangioplasty restenosis in regard to SMC abundance (scale, 0 to 3; 1.4±0.9 versus 1.7±0.9; P=NS), and both differed from those of stable angina. Thrombus and/or hemorrhage occurred in only 34% of patients with unstable angina (compared with 8% of restenosis patients and in none of stable angina patients). Active lesions (defined as lesions containing one or more of the following: thrombus, hemorrhage, abundant and disorganized SMCs in the presence of loose connective tissue, or inflammatory infiltrate) were observed in 56% of the unstable angina patients and in 50% of the restenosis patients but in none of the stable angina patients. The expression of aFGF and bFGF was detected in 80% to 100% of unstable angina (n=11) and restenosis (n=10) specimens but in only 1 of 5 stable angina specimens.**

Conclusions. **Microscopic evidence of thrombosis and plaque rupture occurred in only one third of unstable angina patients, selected because they had no angiographic evidence of intracoronary thrombus. Moreover, their lesions resembled those of restenosis patients in regard to SMC abundance, lesion activity, and the expression of aFGF and bFGF. Our findings therefore suggest that an alternative mechanism to plaque rupture and thrombus formation may be operative in the precipitation of unstable angina; namely, in a subset of patients, SMC proliferation may lead to gradual plaque expansion and thereby to lumenal narrowing and unstable angina. Our data also suggest a role for aFGF and bFGF in this process. (*Circulation*. 1993;88:2493-2500.)**

KEY WORDS • smooth muscle cells • angina • growth factors

Unstable angina pectoris is a major cause of morbidity and mortality leading to 750 000 hospitalizations annually in the United States alone.[1] Thrombosis and primary plaque rupture have been implicated as the mechanisms responsible for the transformation of asymptomatic stable coronary lesions to symptomatic unstable lesions; however, definitive histopathological evidence has been available only in a subgroup of patients with fatal unstable angina pectoris.[2-4] It is therefore possible that other mechanisms may also contribute to the precipitation of unstable angina.

One such mechanism was suggested to us by studies on the pathogenesis of postangioplasty restenosis, a condition that shares the rapid but usually not precipitate development of clinical signs of increasing coronary obstruction. Because smooth muscle cell proliferation has been shown to be a primary causal mechanism in the restenosis process,[5,6] in the present investigation we

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From the Cardiology Branch (M.Y.F., R.C., Z-X Y., Y-M F., W.C., S.E.E.), National Heart, Lung, and Blood Institute, Bethesda, Md; the Division of Cardiology (M.B.L.), Washington Hospital Center, Washington, DC; Cardiac Pathology (R.V., A.F.), the Armed Forces Institute of Pathology, Washington, DC; and the Division of Cardiothoracic Surgery (A.E.), UCLA Medical Center, Los Angeles, Calif.

Correspondence to Dr S.E. Epstein, Cardiology Branch, NHLBI, Building 10, Room 7B-15, Bethesda, MD 20892.

TABLE 1. Demographic and Angiographic Data

Group	Age, Mean (Range)	Sex	Vessels Diseased, No.	Atherectomy Site
Stable AP (n=10)	66 y (53-77)	Male, 10 Patients	1, 5 Patients	LAD, 5 Patients
		Female, 0 Patients	2, 2 Patients	Cx, 2 Patients
			3, 3 Patients	RCA, 2 Patients
				Other, 1 Patient
Unstable AP (n=32)	61 y (41-76)	Male, 27 Patients	1, 15 Patients	LAD, 20 Patients
		Female, 5 Patients	2, 12 Patients	Cx, 6 Patients
			3, 5 Patients	RCA, 3 Patients
				Other, 3 Patients
Restenosis (n=24)	59 y (34-80)	Male, 20 Patients	1, 10 Patients	LAD, 13 Patients
		Female, 4 Patients	2, 10 Patients	Cx, 2 Patients
			3, 4 Patients	RCA, 7 Patients
				Other, 2 Patients

AP indicates angina pectoris; LAD, left anterior descending coronary artery; Cx, circumflex coronary artery; and RCA, right coronary artery.

examined the hypothesis that a similar mechanism is responsible for the development of nonfatal unstable angina pectoris.

To estimate the importance of smooth muscle cell proliferation in the development of this clinical syndrome, we compared atherectomy specimens of lesions of unstable angina patients with those of restenosis patients, whose relatively cellular lesions are known to be composed predominantly of smooth muscle cells,[5-8] and with those of stable angina patients, whose lesions are composed of dense collagen and contain relatively few smooth muscle cells in the fibrous cap.[9] As an integral part of this concept, we also sought to determine the relative abundance in these lesions of both acidic and basic fibroblast growth factors (FGF), as both are important mediators of smooth muscle cell proliferation and migration.[10-13]

Methods

Patients

Atherectomy specimens from 70 consecutive patients undergoing directional coronary atherectomy were analyzed. The patients were referred to a tertiary referral center (Washington Hospital Center) for angiographic diagnosis and therapy. Patients with evidence of significant coronary narrowing (>60% narrowing of a major epicardial artery) and lesion anatomy favorable for directional atherectomy were included in the study. Patients with total coronary occlusion and those with unequivocal angiographic diagnosis of intracoronary thrombus underwent different revascularization procedures and thus were not part of the current investigation. Three patients were excluded from the study because their atherectomy specimens contained only media or were too small to be informative. A fourth patient was excluded from the study because a consensus in regard to the pathological findings could not be reached. Thus, the study consisted of a total of 66 patients.

Patients were classified according to their admission diagnosis into one of the three following groups: (1) unstable angina pectoris (32 patients), defined as one of three clinical syndromes: angina pectoris occurring at rest (17 patients), recent onset angina pectoris (<2 months' duration) (10 patients), and accelerated angina pectoris (5 patients), (2) postangioplasty restenosis (24 patients), defined by atherectomy being performed at least 1 week after angioplasty (mean, 4 months; median, 2 months; range, 1 week to 19 months), and (3) stable angina pectoris (10 patients).

Tissue Preparation

Atherectomy specimens were fixed at the time of the procedure in 10% buffered formalin. Tissue was dehydrated in graded series of alcohol and embedded in paraffin block. Serial sections were stained for hematoxylin and eosin, Movat's pentachrome, Mallory's phosphotungstic acid hematoxylin (PTAH), and Masson's trichrome stains.[14] Serial unstained sections were used for immunohistochemistry.

Immunohistochemistry

In 26 atherectomy specimens, immunohistochemistry was performed using polyclonal antibodies against acidic and basic FGFs. Anti-basic FGF_{1-24} IgG was a kind gift from Dr A. Baird, La Jolla, Calif (concentration used, 2.0 μg/mL), and the antiacidic FGF_{50-82} was a kind gift from Dr J. Sasse, Tampa, Fla (concentration used, 2.5 μg/mL). Both antibodies have been described previously.[15] Specimens were incubated with the primary antibody overnight at 4°C. Incubation with biotinylated secondary antibody was carried out at room temperature, followed by incubation with avidin and biotinylated horseradish peroxidase complex (ABC method, Vector Labs). The sections were counterstained with methyl green. Two controls were used: (1) nonimmune rabbit serum and (2) antibodies preadsorbed with acidic or with basic recombinant human FGF. Due to the small amount of tissue retrieved by coronary atherectomy, the specificity of the antibodies to human acidic and basic FGFs was assessed by Western blotting of protein extracts from four human

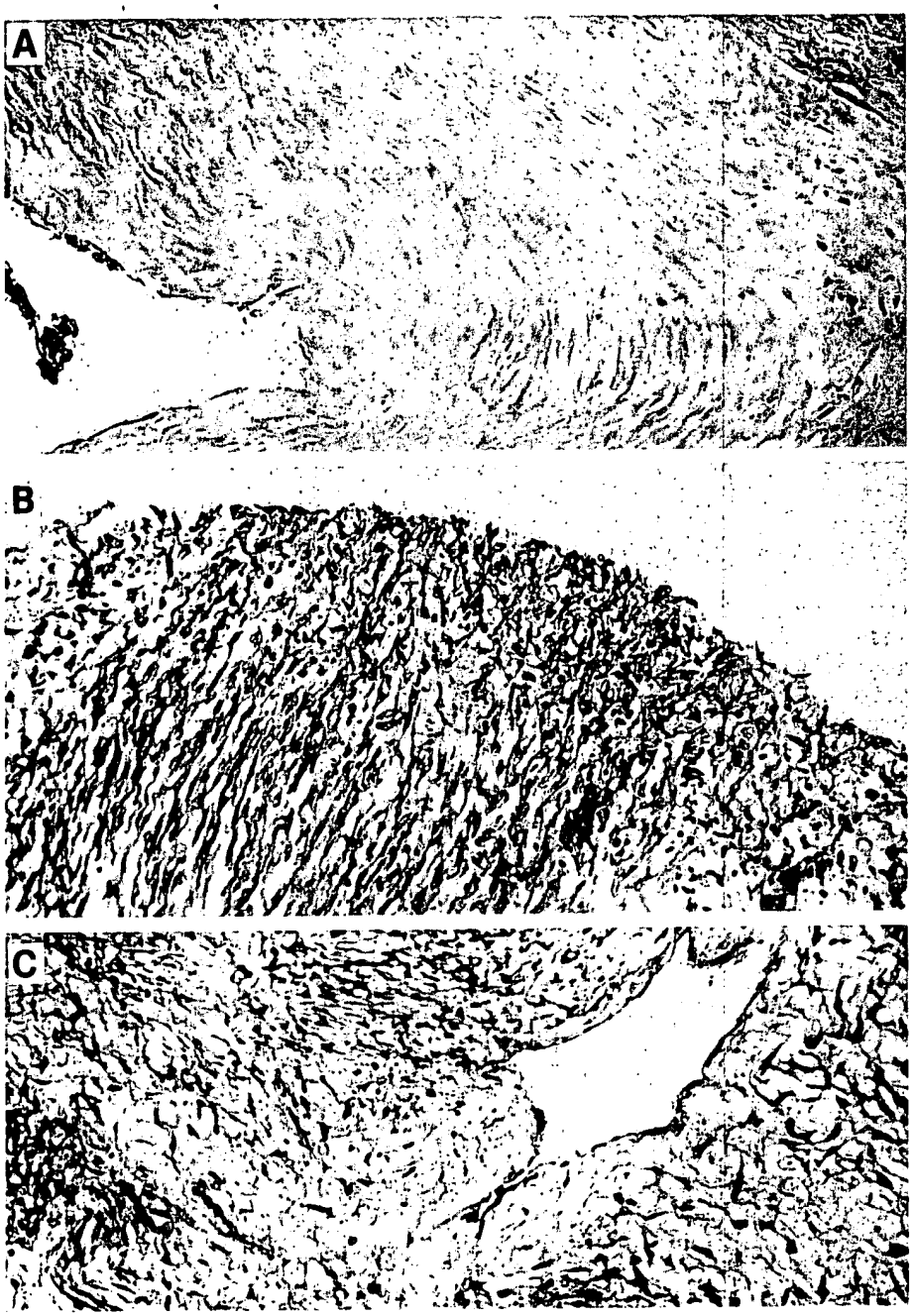

FIG 1. A, Atherectomy specimen from a patient with stable angina pectoris. The few cells evident are separated by dense collagen. B, Atherectomy specimen from a patient with unstable angina pectoris. Note the hypercellularity, the disorganization exhibited by the smooth muscle cells, the loose connective tissue, and the presence of inflammatory cells. C, Atherectomy specimen from a patient with postangioplasty restenosis. The similarity to the specimen of the unstable angina patient is apparent. Hematoxylin and eosin stain; magnification ×160.

coronary arteries. The four arteries were excised from the hearts of patients undergoing heart transplantation. The underlying cause of transplantation was ischemic cardiopathy (2 patients) and dilated cardiomyopathy (2 patients). The arteries were frozen in liquid nitrogen after adjacent tissue was trimmed, and 200 mg of arterial segments was homogenized and proteins were extracted from the homogenate. The extracted proteins were incubated with heparin-Sepharose beads for 18 hours at 4°C. At the end of the incubation, the beads

TABLE 2. Histological Findings

Group	Thrombus and/or Hemorrhage	Active Lesions	SMC Predominance Scale 0 to 3 (mean±SD)
Stable AP (n=10)	0	0	0.7±0.6
Unstable AP (n=32)	11 (34%)	18 (56%)	1.4±0.9
Post-PTCA restenosis (n=24)	2 (8%)	12 (50%)	1.7±0.9

SMC indicates smooth muscle cell; AP, angina pectoris; and PTCA, percutaneous transluminal coronary angioplasty.

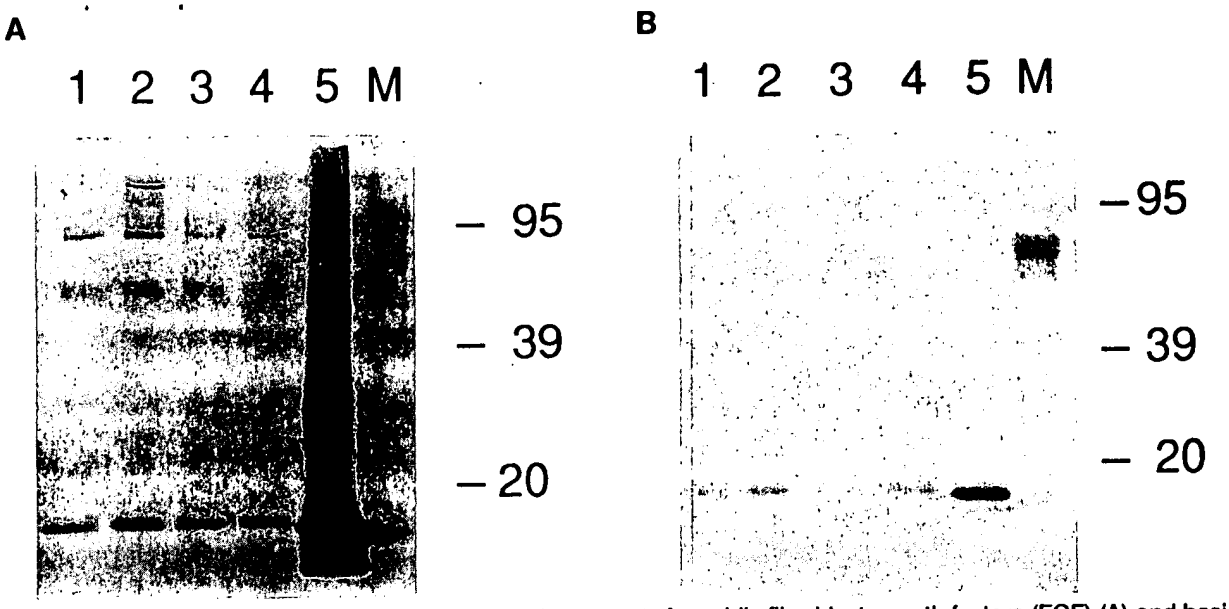

FIG 2. Western blot analysis of human coronary arteries extracts for acidic fibroblast growth factors (FGF) (A) and basic FGF (B). Lanes 1 to 4, extracts from human coronary arteries excised from the diseased hearts of patients undergoing heart transplantation. Lane 5, human recombinant acidic FGF (A) or basic FGF (B) (50 ng); lane M, size markers. Not the distinct 16-18 kD bands in lanes 1 to 5, indicating specific identification of human acidic and basic FGFs by the antibodies used for immunohistochemistry. The higher-molecular-weight bands represent dimers of FGFs.

were washed in 0.6M NaCl and then boiled. The proteins removed from the beads were run in a polyacrylamide gel, with size markers and human recombinant acidic and basic FGF (UBI, Lake Placid, NY) as positive controls in separate lanes. After blotting the samples to nitrocellulose, the blots were hybridized with the antibodies used for immunohistochemistry and developed with anti-rabbit IgG labeled with alkaline phosphatase.

Histochemical and Immunohistochemical Analysis

The stained specimens were analyzed by three independent observers blinded to the patient's clinical diagnosis. The specimens were analyzed for (1) the presence or absence of thrombus and hemorrhage, (2) the presence of smooth muscle cells, based on cell morphology and PTAH staining[16] (graded 0 to 3; 0, absence of smooth muscle cells; 3, predominance of smooth muscle cells in the specimen), and (3) lesion activity, where active lesions were defined as containing one or more of the following: thrombus, hemorrhage, abundant and disorganized smooth muscle cells in the presence of loose connective tissue, or inflammatory infiltrate. The immunohistochemical sections were classified as positive for the presence of acidic or basic FGFs when the cell cytoplasm stained brown and negative when no peroxidase reaction was noted. Because we previously found a good correlation between PTAH staining and immunohistochemistry for α-smooth muscle cell actin for the identification of smooth muscle cells in atherectomy specimens, we used PTAH staining in the present investigation to assess predominance of smooth muscle cells.[17]

Statistical Analysis

To compare the rating of smooth muscle cell predominance, we used the Mann-Whitney test. For dichotomous variables, we used Fischer's exact or χ^2 tests.

Results

The demographic and angiographic data of patients are summarized in Table 1. The three observers agreed in 89% of cases in regard to plaque hemorrhage, 78% of cases in regard to the presence of thrombus, and in 88% of cases with regard to lesion activity. In cases of disagreement, the opinion of the majority was used in the analysis. For smooth muscle predominance, the arithmetical average was used in the analysis.

Typical lesions of patients with stable angina pectoris, unstable angina pectoris, and postangioplasty restenosis stained with hematoxylin eosin are shown in Fig 1, and the histological findings in the three groups of patients are summarized in Table 2. Analysis of the atherectomy specimens of patients with unstable angina pectoris demonstrated that while only a minority (34%) of the specimens had evidence of thrombus or hemorrhage, the prevalence of this finding was still significantly higher than in the specimens of patients with restenosis (8%) ($P<.03$) or of those with stable angina (0%). Active lesions were observed in about half of both the unstable angina (56%) and in restenosis patients (50%) but in none of the stable angina patients. Smooth muscle cells predominated in the specimens of both patients with restenosis and those with unstable angina (1.7 ± 0.9 versus 1.4 ± 0.9, P=NS), whereas the lesions of patients with stable angina showed far fewer smooth muscle cells (0.7 ± 0.6).

Western blot analysis (Fig 2) demonstrated that the antibodies used in the immunohistochemical analysis recognized acidic and basic FGFs, as indicated by the positive immunoreaction with heparin binding proteins extracted from human coronary arteries; these proteins were of the identical molecular weight as human recombinant acidic and basic FGFs.

Typical immunohistochemical findings of unstable angina patients using antibodies directed against acidic

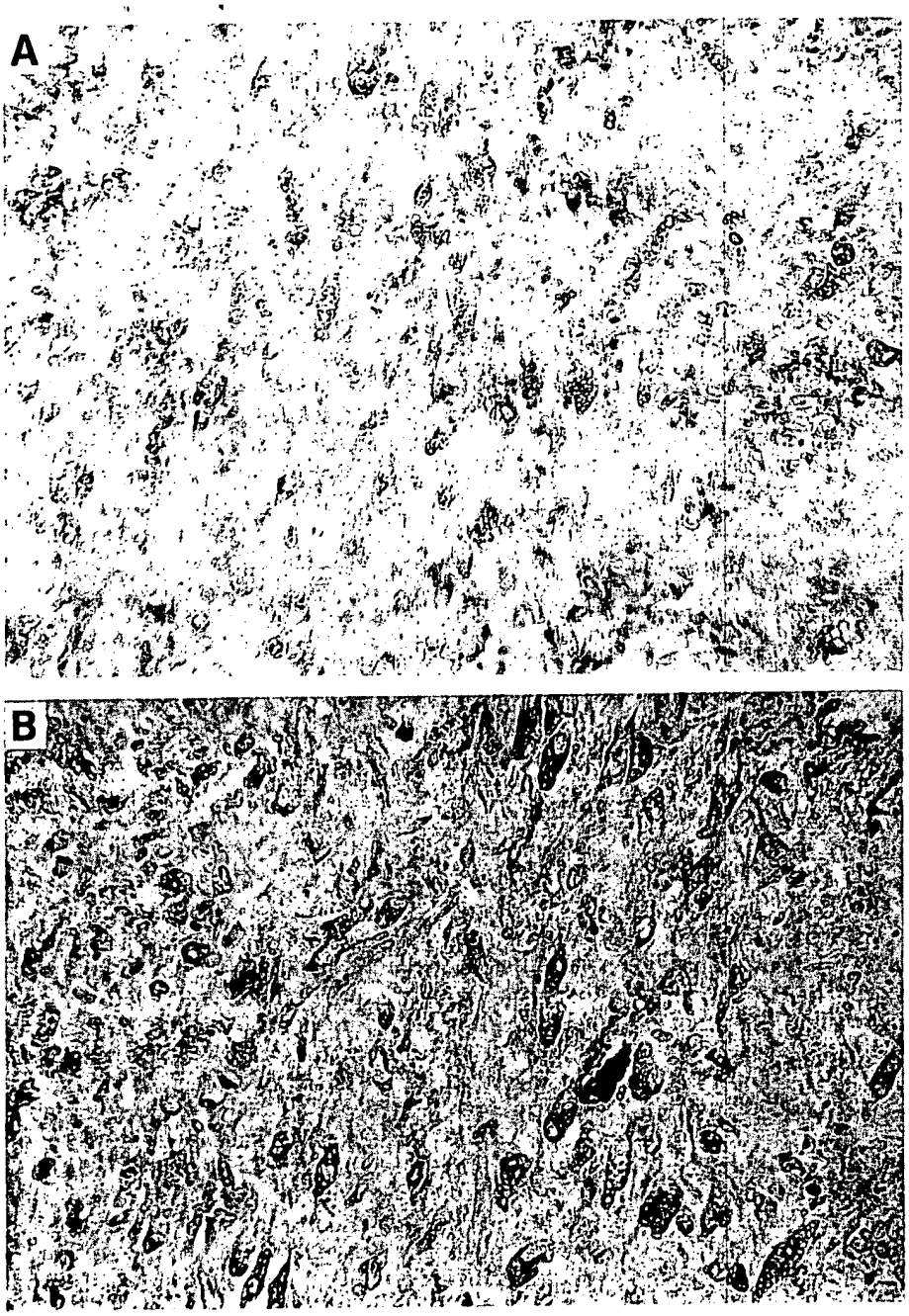

FIG 3. Immunohistochemistry of an atherectomy specimen from a patient with unstable angina. In panel A (magnification ×250), the positive peroxidase reaction (immunoreactive acidic fibroblast growth factor, FGF), demonstrated by the brown stain, is localized to the cytoplasm. The nuclei are counterstained green. Most of the cells in the specimen are immunoreactive to acidic FGF. In panel B (magnification ×400), most cells are immunopositive for basic FGF.

FGF and basic FGF are demonstrated in Figs 3A and 3B, respectively. Analysis of the immunohistochemical staining showed that immunoreactivity for acidic and basic FGFs was observed in most patients with unstable angina and restenosis and in only 1 out of 5 in the stable angina group (20%) (Figs 4A and 4B).

Discussion

Previous studies designed to investigate the mechanisms responsible for the development of unstable angina pectoris have concluded that the clinical syndrome is caused by plaque rupture, hemorrhage, and thrombus formation.[2-4] This conclusion derives from studies using post mortem analyses,[2-4] coronary angiography,[18-23] and coronary angioscopy,[24,25] which convincingly proved the validity of this causal linkage. The presence of thrombus and the contribution of dynamic changes of vascular tone (as suggested by experimental observations of cyclic flow variations) undoubtedly explains the clinical course of many patients with unstable angina pectoris.[26] However, many of these studies demonstrated that a sizeable percentage of patients with unstable angina do not have plaque rupture or thrombus that can be identified, at least at the time of the studies. Moreover, only a minority of patients with unstable angina pectoris will respond favorably to thrombolytic therapy.[27] Hence, it would appear that plaque rupture and thrombus formation are not the only mechanisms leading to the precipitation of unstable angina.

In the present investigation, almost two thirds of our patients exhibited no evidence of thrombus on analysis of

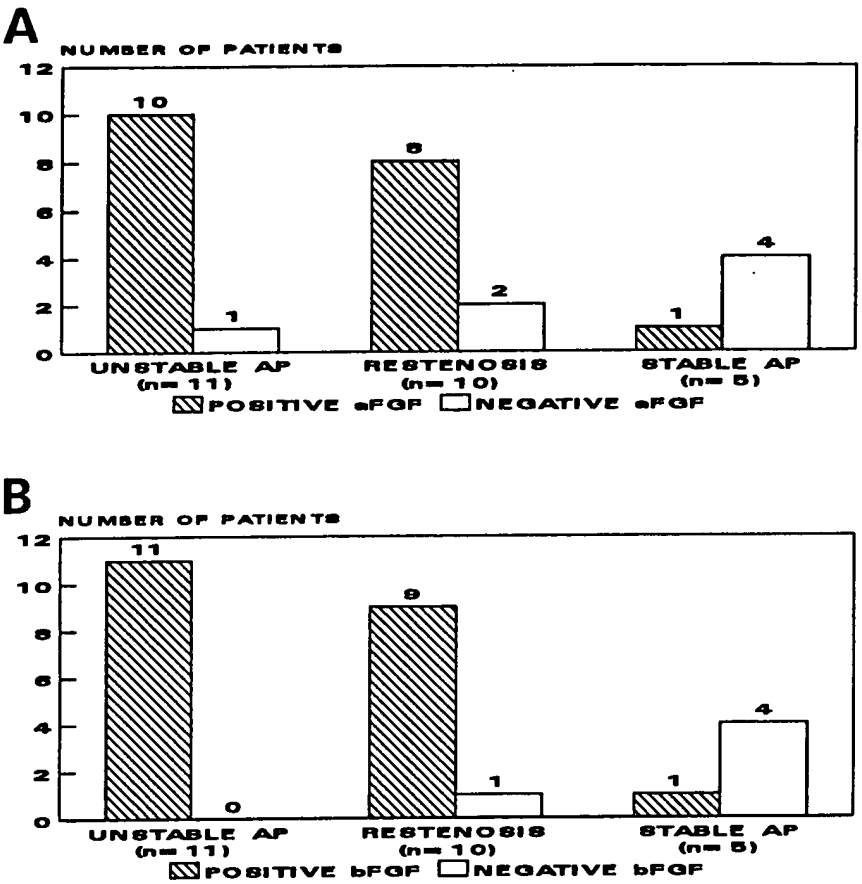

FIG 4. Bar graphs show classification of the atherectomy specimens with regard to the presence (positive) or absence (negative) of acidic (A) or basic (B) fibroblast growth factors (FGF) in the three groups of patients. AP indicates angina pectoris.

tissue derived from atherectomy. This figure underestimates the prevalence of thrombus in unstable angina because only patients who had no evidence of intraluminal thrombus on angiography were entered into the study. The fact remains, however, that there is still a significant number of patients with unstable angina, in this and other studies, who have no angiographic or pathological evidence of intracoronary thrombus.[18-23,28,29]

It must be pointed out that by the time of atherectomy in this subgroup of patients, it is possible the original plaque dissection had healed, and any thrombus originally present had lysed or organized. Hence, plaque rupture and thrombus formation cannot be definitively ruled out as the common cause of all episodes of unstable angina pectoris. Moreover, the size of atherectomy specimens is small, and it can be argued that the apparent lack of thrombus was due to sampling error. The stable angina group is rather small and serves mostly to amplify the similarities between the groups of unstable and restenosis patients.

Although our study cannot refute such possibilities, the results do provide an alternative mechanism to plaque rupture, hemorrhage, and thrombus formation in the precipitation of unstable angina in a subset of patients. Thus, in the majority of the specimens obtained from patients with unstable angina, the bulk of the lesions consisted of cells in a loose extracellular matrix (predominantly glycosaminoglycans); moreover, smooth muscle cells were the dominant cell type. Such findings rendered these specimens indistinguishable from those of patients with restenosis. This observation is conceptually important because human and animal studies have provided evidence that arterial injury induces smooth muscle proliferation and migration with the production of loose connective tissue and that this mechanism contributes to postangioplasty restenosis. The fact that the histological characteristics of the lesions of patients undergoing atherectomy for unstable angina pectoris are indistinguishable from those of patients with restenosis strongly suggests that the mechanism responsible for both may be the same: Smooth muscle proliferation and the associated secretion of glycosaminoglycans increase the mass of the atheroma, which thereby exacerbates the coronary obstruction and precipitates an ischemic syndrome.

Our hypothesis is further supported by the finding that the expression of both acidic and basic FGFs are prominent in the lesions derived from unstable angina patients when compared with the expression of these peptides in patients with stable angina. The lesions of patients with stable angina were also relatively acellular (we must emphasize, however, that our stable angina group is too small to make such comparisons definitive).

Just as the histological appearance of the unstable angina lesion was similar to that of the restenosis lesion, so was the immunohistochemical appearance; both displayed high levels of expression of acidic and basic FGFs. Acidic and basic FGF have been found to stimulate proliferation and migration in many cell types, including smooth muscle cells, both in vitro and in vivo.[10,30-32] The presence of the growth peptides should be regarded as an indicator to the activity of the lesions

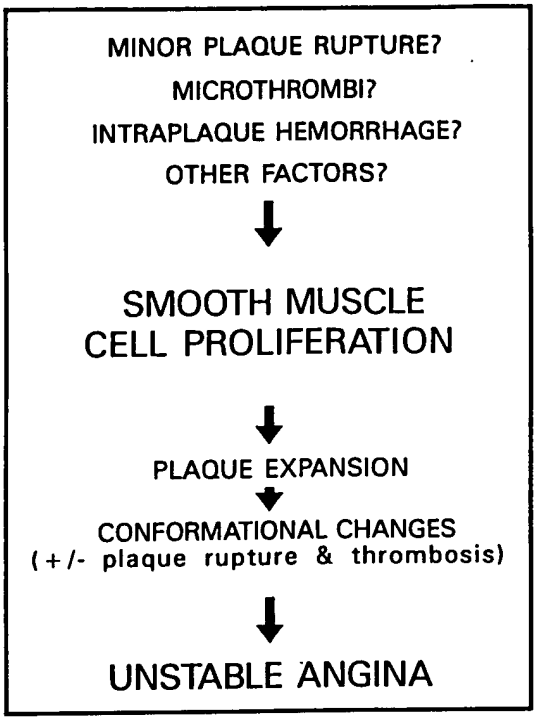

FIG 5. Proposed scheme for the pathophysiological mechanism causing unstable angina in patients in whom major plaque rupture and thrombosis do not play a major role.

and should not carry any implications regarding their role in the triggering events of unstable transformation.

We wish to emphasize that our findings do not negate the prevailing concept that unstable angina occurs as a result of plaque rupture and thrombus formation. We believe that these mechanisms undoubtedly account for the precipitation of unstable angina in many patients.[26,33-36] This concept is supported by the findings of histological evidence of thrombus and/or hemorrhage in a significant number of patients, even in our selected group of patients. Our findings do not negate the possibility that changes in vascular tone contribute to the development of unstable angina (as a primary cause or by triggering the development of plaque rupture or thrombus formation). On the other hand, our data support the concept that the precipitation of unstable angina cannot be ascribed to this mechanism alone. Rather, it appears that its pathophysiology is more complex and that one of the additional contributing causes is smooth muscle cell proliferation, a process that may be amplified, at least in part, by acidic and basic FGFs.

This conceptualization, even if correct, does not identify the primary precipitating stimulus leading to overexpression of acidic and basic FGFs and to smooth muscle cell proliferation. We can at this time only speculate as to the possible triggering event. Thus, it is possible that hemorrhage into a plaque, minor fibrous cap tears and dissection, microthrombi with dynamic changes of vascular tone, or other mitogenic stimuli lead to the expression of multiple growth factors, including acidic and basic FGFs, which in turn initiate a cascade of events in which the dominant component is smooth muscle cell proliferation (Fig 5). This also may be associated with migration of smooth muscle cells from the underlying media into the plaque and the synthesis and secretion by smooth muscle cells of extracellular matrix, processes leading to expansion of the original plaque. Given the complexity of the process, it is also possible that the expansion and resulting conformational changes caused by this proliferative mechanism may make the plaque more vulnerable to ulceration and secondary thrombus formation and that in some patients, both of these mechanisms contribute to the precipitation of unstable angina.

Conclusions

We believe that the development of unstable angina is precipitated by plaque rupture and thrombus formation in many individuals, but in others it may be caused by excessive smooth muscle cell proliferation. Although we cannot yet identify the mechanisms that trigger smooth muscle cell proliferation in patients whose clinical situation changes from a stable to an unstable anginal pattern, our findings will, we hope, lead to future studies designed to elucidate the responsible mechanisms. Such information, once obtained, will undoubtedly improve our approach to the treatment and perhaps to the prevention of the development of unstable angina pectoris.

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serve Local Rule 12 and permit oral argument on defendant's Motion for Summary Judgment deprived movant of its right to be heard and raises a serious question whether due process was ignored.

Because the constitutionality of a federal statute was drawn in question, the United States intervened pursuant to 28 U.S.C. §203 (1982), and has participated in this appeal.

In a short memorandum the magistrate rejected both of these contentions and denied relief from the judgment. Auld appealed to the Court of Appeals for the Sixth Circuit. That court transferred the case to this court on the ground that "a Rule 60(b) motion is a continuation of the original action" and that this court "has exclusive appellate jurisdiction over the instant appeal." The parties do not challenge that ruling, and we agree that we have jurisdiction.

II

Eight circuit courts of appeals, including two in banc, have now upheld the constitutionality of the consensual reference procedures of the Federal Magistrates Act of 1979, and the Supreme Court three times had declined to review those rulings. Fields v. WMATA, No. 83-1186 (D.C. Cir. Sept. 11, 1984); Goldstein v. Kelleher, 728 F.2d 32 (1st Cir. 1984), cert. denied, 3 U.S.L.W. 3239 (U.S. Oct. 1, 1984) (No. 84-5); Collins v. Foreman, 729 F.2d 108 (2d Cir. 1984), cert. denied, 53 U.S.L.W. 3240 (U.S. Oct. 1, 1984) (No. 83-1616); Puryear v. Ede's Ltd., 731 F.2d 1153 (5th Cir. 1984); Geras v. Lafayette Display Fixtures, Inc., 742 F.2d 1037 (7th Cir. 1984); Lehman Brothers Kuhn Loeb, Inc. v. Clark Oil & Refining Corp., 739 F.2d 1313 (8th Cir. 1984) (in banc), petition for cert. filed, 53 U.S.L.W. 3291 (U.S. Sept. 29, 1984) (No. 84-519); Pacemaker Diagnostic Clinic, Inc. v. Instromedix, Inc., 725 F.2d 537 (9th Cir. 1984) (in banc), rev'g 712 F.2d 1305, 220 USPQ 502 (9th Cir. 1983), cert. denied, 53 U.S.L.W. 3236 (U.S. Oct. 1, 1984) (No. 83-1783) (patent infringement suit); and Wharton-Thomas v. United States, 721 F.2d 922 (3d Cir. 1983).

Auld has offered no convincing ground to reject those decisions, and we cannot discern any. Although the Sixth Circuit, in which this case arose, has not decided the question, there is no reason to believe that it would disagree with the eight circuits that have upheld the statute.

In view of the extensive and convincing analysis of the constitutional question in those opinions, it is unnecessary to discuss the issues at any length. Auld relies largely upon the panel decision of the Ninth Circuit in Pacemaker Diagnostic Clinic which, as Auld recognizes, that court reversed in its in banc decision. It is hardly necessary to point out that an overruled decision neither states the law nor is an appropriate source for determining it.

Under the Magistrates Act the district court appoints the magistrates, authorizes them to conduct civil proceedings, and authorizes each particular reference which, as noted, may be made only with the consent of the parties. The district court may revoke a reference, and only it may punish contempts committed before a magistrate. The magistrate's decision may be appealed to the court of appeals or, by advance agreement of the parties, to the district court. 28 U.S.C. §636(c).

The arguments sustaining the constitutionality of these provisions are well summarized in the following statement in Goldstein v. Kelleher, 728 F.2d at 36, with which we agree:

> [T]he Article III interests of both the litigants and the judiciary are adequately protected under section 636(c)(3) * * * * The litigants' interests are safeguarded by the consensual nature of the reference; the institutional interests of the judiciary are secured by the district court's control over both the references and appointments, and by the availability of appeal to an Article III court.

III

A. Auld argues that the failure of the magistrate to grant its request for an oral hearing before he entered summary judgment required grant of its Rule 60(b) motion because such failure invalidated the summary judgment. That question was fully litigated and considered in the prior appeal in this case. Our opinion there discussed the point at some length and fully explained why the failure provided no basis for reversal of the summary judgment. 714 F.2d at 1151-52, 219 USPQ at 19.

That prior decision was the law of the case. Gindes v. United States, 740 F.2d 947 (Fed. Cir.), cert. denied, No. 84-737 (Dec. 3, 1984). Auld does not ever refer to that principle and makes no attempt to bring this case within the only possible exception to it, namely, that "the prior decision 'was clearly erroneous and would work a manifest injustice.'" Gindes, 740 F.2d at 950.

B. Auld also argues that in consenting to the reference to the magistrate it agreed to a reference only for a trial but not for disposition by summary judgment. Since Auld did not raise this point in its Rule 60(b) motion, the issue is not properly before us. Moreover, Auld offers no reason why it did not make the argument in its prior appeal.

Finally, the contention is frivolous. Auld consented to have the magistrate "conduct any and all further proceedings in this case, including trial, and order the entry of a final judgment." The specific reference to "trial" was designed to show the breadth of the magistrate's authority, not to limit his power. The summary judgment the magistrate entered was part of the "further proceedings in this case" he was empowered to conduct and was "a final judgment" he was authorized to enter.

IV

[1] Section 285 of the Title 35, U.S.C. (1982), provides: "The court in exceptional cases may award reasonable attorney fees to the prevailing party." This provision authorizes us to award to the prevailing party before this court its attorney's fees incurred in its successful handling of an appeal. See Shelcore, Inc. v. Durham Industries, Inc., 745 F.2d 621, 629-30, 223 USPQ 584, 591 (Fed. Cir. 1984); Rohm & Haas Co. v. Crystal Chemical Co., 736 F.2d 688, 222 USPQ 97, 100 (Fed. Cir.), cert. denied, 53 U.S.L.W. 3239 (U.S. Oct. 1, 1984) (No. 84-1).

This is an exceptional case in which an award of attorney fees to the appellee is warranted.

When Auld filed its opening brief in this court on August 27, 1984, seven circuits already had upheld the constitutionality of the provision Auld challenges. These included the in banc decision of the Ninth Circuit in Pacemaker Diagnostic Clinic, which overruled the panel decision upon which Auld heavily relied. Those opinions were rendered 4 days (Geras), 37 days (Lehman Bros.), almost 4 months (Puryear), approximately 6 months (Goldstein, Collins, and Pacemaker Diagnostic Clinic), and 21 months (Wharton-Thomas) before Auld filed its brief. Auld either was or should have been aware of at least six of them.

Auld contended that Northern Pipeline Construction Co. v. Marathon Pipe Line Co., 458 U.S. 50 (1982), which held unconstitutional provisions of the Bankruptcy Act of 1978 that authorized bankruptcy judges to perform certain functions of Article III judges, invalidated §636(c) of the Magistrates Act. The courts of appeals that upheld the constitutionality of §636(c) also had considered but rejected the argument based upon Northern Pipeline.

In short, when Auld filed its opening brief it had no reasonable basis for believing that its constitutional argument had any likelihood of prevailing before this court.

Auld's contention based upon the magistrate's failure to hold an oral hearing before granting summary judgment had been rejected in the prior appeal. Auld showed no awareness that that decision was the law of the case and made no attempt to show that his case was within one of the narrow exceptions to that doctrine.

Finally, Auld's argument that it did not consent to a reference to the magistrate to decide the case on summary judgment was not even presented in Auld's Rule 60(b) motion and, in any event, was frivolous.

In sum, Auld's pursuance of this appeal was, as was the appeal in Colt Industries Operating Corporation v. Index-Werke K.G., 739 F.2d 622, 623 (Fed. Cir. 1984), "abusive of the judicial process."

In the prior appeal, Auld fully litigated but lost the argument that summary judgment holding its patent invalid was improper. Instead of accepting that decision or seeking further review in the Supreme Court, Auld attempted to escape that decision by seeking to reopen the judgment of the district court on what turned out to be insubstantial grounds. When that effort failed, Auld persisted in pursuing an appeal that had no chance of success. In the circumstances the appellee is entitled to recover from Auld the attorney's fees it incurred in its successful defense against the appeal.

Conclusion

The order of the district court entered by the United States magistrate denying Auld's Rule 60(b) motion is affirmed. Auld shall reimburse the appellee Chroma for the attorney's fees the latter incurred in handling this appeal.

Affirmed.

Court of Appeals, Federal Circuit

Cross et al. v. Iizuka et al.

No. 84-111

Decided Jan. 28, 1985

PATENTS

1. Patentability — Utility (§51.75)

Board did not err in finding that in vitro utility disclosed in foreign priority application

is sufficient to establish practical utility under 35 USC 101.

2. Patentability — Utility (§51.75)

Rigorous correlation of pharmacological activity between disclosed in vitro utility and in vivo activity is not necessary where disclosure of pharmacological activity is reasonable based upon probative evidence.

3. Patentability — Utility (§51.75)

35 USC 112 "how to use" requirement is satisfied, despite failure of disclosure to reveal dosages for novel compound per se, those skilled in art having had sufficient information at critical date to determine dosage for desired pharmacological activity.

Particular patents — Imadazole Derivatives

Iizuka, et al., application, Imidazole Derivatives, award of priority over Cross et al., application, N-(Phenoxyalkyl) Imidazoles as Selective Inhibitors of the Thromboxane Synthetase Enzyme and Pharmaceutical Compositions Thereof, affirmed .

Appeal from Patent and Trademark Office Board of Patent Interferences.

Patent interference No. 100,650, between Peter E. Cross, et al., application, Serial No. 95,755, filed Nov. 19, 1979, and Kinji Iizuka, et al., application, Serial No. 68,365, filed Aug. 21, 1979. From decision awarding priority to party Iizuka, party Cross, et al. appeals. Affirmed.

Rudolf E. Hutz, and Connoly, Bove, Lodge & Hutz, both of Wilmington, Del. (Thomas M. Meshbesher, Wilmington, Del., on the brief) for appellants.

Peter D. Olexy, and Sugrue, Mion, Zinn, MacPeake & Seas, both of Washington, D.C. (Thomas J. MacPeak, Washington, D.C., on the brief) for appellees.

Before Kashiwa, Bennett, and Bissell, Circuit Judges.

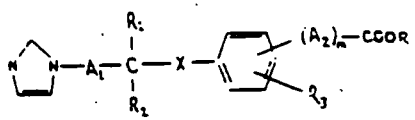
Kashiwa, Circuit Judge.

This appeal is from the decision of the United States Patent and Trademark Office (PTO) Board of Patent Interferences (Board) awarding priority on the single phantom count to Iizuka, et al. (Iizuka), the senior party. We affirm.

Background

Interference No. 100,650 was declared on 20 April 1981 between application serial No. 68,365, for "Imidazole Derivatives," filed by Iizuka on 21 August 1979 and application serial No. 95,755, for "N-(Phenoxyalkyl) Imidazoles as Selective Inhibitors of the Thromboxane Synthetase Enzyme and Pharmaceutical Compositions Thereof," filed by Cross, et al. (Cross) on 19 November 1979. The single phantom count of the interference is directed to imidazole derivative compounds and reads as follows:

A compound selected from the group consisting of an imidazole derivative of the formula



wherein R is a hydrogen atom or an alkyl group having 1 to 6 carbon atoms, each of A_1 or A_2, which may be the same or different, are alkylene having 1 to 8 carbon atoms, m is 0 or 1, X is oxygen or sulfur, and each of R_1 or R_2, which may be the same or different, is a hydrogen atom *or an alkyl group having 1 to 6 carbon* atoms; R_3 is H, C_1-C_4 alkyl, C_1-C_4 alkoxy or halogen; and the pharmaceutically acceptable salts thereof.[1]

The applications of Cross and Iizuka both disclose inventions directed to imidazole derivative compounds which inhibit the synthesis of thromboxane synthetase, an enzyme which leads to the formation of thromboxane A_2 (TXA_2)[2] a highly unstable, biologically active compound which is converted to stable thromboxane B_2 by the addition of water. Thromboxane A_2, as of the time period during which the applications were filed, was postulated to be a causal factor in platelet aggregation.[3] Platelet aggregation is associated with several deleterious conditions in mammalia, including humans, such as platelet thrombosis, pulmonary vasoconstriction or vasospasm, inflammation, hypertension, and collagen-induced thrombosis.

Pursuant to 37 C.F.R. §1.231(a)(4) each party moved to be accorded the benefit of a foreign priority application under 35 U.S.C. §119, Cross claiming priority based upon a British application filed 13 December 1978, and Iizuka claiming priority based upon a Japanese application filed 21 August 1978. Each party opposed the motion of the other party, each party contending that the other party's foreign priority application did not comply with the disclosure requirements of 35 U.S.C. §112.

The primary examiner granted each party's motion, noting that the utility alleged in each application was of a pharmacological nature, i.e., the inhibition of thromboxane synthetase, and that inasmuch as the single phantom count of the interference was directed to a compound, it was not necessary that utility be established by tests and dosages with respect to human beings. The examiner found that one of ordinary skill in the art would know how to use the imidazole derivatives, i.e., be able to determine specific dosages, for biological purposes. Based upon the filing dates of the foreign priority applications,[4] Iizuka was declared the senior party and a show cause order was issued against Cross.

Cross requested a final hearing on the issue of the sufficiency of the Japanese priority application of Iizuka, and moved for a testimony period to present evidence on this issue. A testimony period was granted over the opposition of Iizuka, and Cross took the testimony of his expert witness, Dr. Smith, and Iizuka took the testimony of his expert witness, Dr. Ramwell and also proferred several exhibits pursuant to 37 C.F.R. §1.282. All testimony and exhibts related to the sufficiency of Iizuka's Japanese priority application, i.e., whether it complied with the disclosure requirements of 35 U.S.C. §112.

Decision of the Board

The Board noted that the sole issue before it was whether Iizuka was entitled to the benefit of his Japanese priority application.[5] Relying on In re Bundy, 642 F.2d 430, 209 USPQ 48 (CCPA 1981), and Nelson v. Bowler, 626 F.2d 853, 206 USPQ 881 (CCPA 1980), the Board held that tests evidencing pharmacological activity may manifest a practical utility even though they may not establish a specific therapeutic use. The Board found that the Japanese priority application disclosed pharmacological activity in the similar activity of the imidazole derivatives of the count to imidazole and 1-methylimidazole, which possess an inhibitory action for thromboxane synthetase, and that practical utility was disclosed in the strong inhibitory action for thromboxane synthetase from human or bovine platelet microsomes, i.e., an in vitro utility.[6]

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[1] We note a discrepancy, shown underlined in the above count, between the phantom count as set forth by the primary examiner and that reported by the Board in its decision. The phantom count set forth herein is the one propounded by the primary examiner. However, as will become apparent from the ensuing discussion, the substance of the phantom count is not crucial to resolution of the issues presented by this case.

[2] The formation of TXA_2 in an arachidonic acid challenge is a sequential process initiated by the conversion of arachidonic acid to postaglandin PGG_2 by the action of cyclooxygenase, which adds oxygen to the acid. Peroxidase converts the prostaglandin PGG_2 to prostaglandin PGH_2, which in turn is converted by thromboxane synthetase to TXA_2.

[3] Iizuka's position is that, as of the "critical date" of his application, TXA_2 was widely accepted in the art as causing platelet aggregation. Cross' position is that, as of the "critical date," platelet aggregation was believed to be nonspecific, i.e., platelet aggregation *may* occur in the presence of thromboxane synthetase, but thromboxane synthetase is not necessary for platelet aggregation. We note in retrospect that THE MERCK INDEX 1345-46 (10th ed. 1983) describes TXA_2 as inducing irreversible platelet aggregation. More to the point, however, this court has noted that it is axiomatic that an inventor need not comprehend the scientific principles on which the practical effectiveness of his invention rests, nor is the inventor's theory or belief as to how his invention works a necessary element in the specification to satisfy the enablement requirement of 35 U.S.C. §112. Fromson v. Advance Offset Plate, Inc., 720 F.2d 1565, 1570, 219 USPQ 1137, 1140 (Fed. Cir. 1983).

[4] Each party relies on the filing date of its foreign priority application to establish a constructive reduction to practice, the earliest date of invention to which each party is entitled under the patent laws of the United States. Kawai v. Metlesics, 480 F.2d 880, 885-86, 178 USPQ 158, 162 (CCPA 1973).

[5] More specifically, the issue before the Board was whether the Japanese priority application complied with the how-to-use requirement of 35 U.S.C. §112. Section 112 of Title 35 provides, in pertinent part, that:

> The specification shall contain a written description of the invention, of the manner and process of making and *using* it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and *use* the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention. (Emphasis added.)

Should Iizuka's Japanese priority application be found nonenabling with respect to the how-to-use requirement of §112, or otherwise found deficient under the patent laws of the United States, priority would be awarded to Cross based upon his unchallenged entitlement to the benefit of his British application.

[6] Generally, in vitro refers to an environment outside of a living organism, usually an artificial environment such as a test tube or culture. In contradistinction, in vivo generally refers to an environment within a living organism, such as a

The Board further found that the Japanese priority application disclosed "how-to-use" knowledge directed to the practical utility in a microsome system, and that microsome assays were admittedly known in the art. A skilled worker could determine the relative strength of the imidaznle compounds of the count vis-a-vis the known parent imidazole and 1-methylimidazole compounds for use in the microsome assay milieu. Knowledge of the pharmacological activities of compounds is beneficial to the medical profession, and requiring Iizuka to have disclosed in vivo dosages in the Japanese priority application would delay and frustrate researchers by failing to provide an incentive for early public disclosure of such compounds, thereby failing to further the public interest.

Accordingly, the Board held that the Japanese priority application contained an adequate how-to-use disclosure for the practical utility stated therein.

Issues

Whether the Board erred in finding that the utility disclosed in the Japanese priority application is sufficient to meet the practical utility requirement of 35 U.S.C. §101.

Whether the Board erred in finding that the Japanese priority application contained sufficient disclosure to satisfy the enablement, i.e., how-to-use, requirement of 35 U.S.C. §112.[7]

Opinion

Proper resolution of the issues before this court necessitates that we address, seriatim, the following questions: (1) What utility is disclosed by the Japanese priority application? (2) Does this stated utility comply with the "practical utility" requirement of 35 U.S.C. §101, as delimited by prior decisions of the judiciary?[8] (3) Does the Japanese priority application contain sufficient disclosure to meet the how-to-use requirement of §112 with respect to the stated utility?

It is axiomatic that an invention cannot be considered "useful," in the sense that a patent can be granted on it, unless substantial or practical utility for the invention has been discovered and disclosed where such utility would not be obvious. Brenner v. Manson, 383 U.S. 519, 148 USPQ 689 (1966). Where a constructive reduction to practice is involved, as contrasted to an actual reduction to practice, a practical utility for the invention is determined by reference to, and a factual analysis of, the disclosures of the application. Kawai v. Metlesics, 480 F.2d 880, 178 USPQ 158 (CCPA 1973).

1. Japanese Priority Application

The Board factually analyzed the Japanese priority application and found that the only effective disclosure relating to a stated utility for the imidazole derivative compounds of the phantom count was the following:

> [The compounds disclosed] are useful for treatment of inflammation, thrombus, hypertension, cerebral apoplexy, asthma, etc.
>
> Up to this time, it is a known fact that imidazole and 1-methylimidazole posses an inhibitory action for thromboxane synthetase and inhibit a biosynthesis of thromboxane A_2. (Prostaglandins, Vol. 13, pages 611-1977). However, since their inhibitory effect is not satisfactory one, these compounds have not been put to practical use yet as therapeutical medicines for diseases caused by thromboxane A_2, such as inflammation, hypertension, thrombus, cerebral apoplexy, asthma, etc.
>
> To develop some compounds possessing a strong inhibitory action for biosynthesis of thromboxane A_2, the present inventors devoted themselves to study for various imidazole derivatives, and as a result, found that the compounds [of this invention] possess a strong inhibitory action for thromboxane synthetase from human or bovine platelet microsomes and are extremely useful as therapeutically active agents for diseases caused by thromboxane A_2, for example, inflammation, hypertension, thrombus, cerebal apoplexy, asthma, etc., and thus were proposed this invention based upon those findings.
>
> * * * * *
>
> The imidazole derivatives * * * of this invention are novel compounds which are not described in literature, and which possess a strong inhibitory action for thromboxane synthetase from human or bovine platelet microsomes, and which exhibit a strong inhibitory action for biosynthesis of thromboxane A_2 in mammalia including human. In general, a satisfactory inhibitory effect is found at a level of molar concentrations of 2.5×10^{-8}, for example, 2-[p-(1-imidazolylmethyl)phenoxy]-acetic acid hydrochloride produce the about 50% inhibitory effect at the molar concentrations of 2.5×10^{-8}. Accordingly, the imidazole derivatives of this invention are extremely useful as therapeutical medicines for diseases caused by thromboxane A_2, such as inflammmation, hypertension, thrombus, cerebral apoplexy, asthma, etc.

The Board found that these pertinent sections of the Japanese priority application disclosed some activity or utility, namely that the imidazole derivative compounds of the count possess a strong inhibitory action for thromboxane synthetase in human or bovine platelet microsomes. Cross' position is that the stated purpose or *sole* contemplated utility of the invention of Iizuka is to provide a novel class of compounds which provide "practical use" as "therapeutical medicines for diseases caused by thromboxane A_2," and therefore the Board erred in its finding as to the stated utility of the Japanese priority application.

While recognizing that Kawai constrains an applicant to entitlement to the benefit of only what is disclosed in the foreign priority application and no more, we also recognize that foreign priority applications, as subsequently filed in the PTO, typically have a style and format dissimilar to the arrangement of application elements suggested by 37 C.F.R. §1.77. In part this arises because of differences in filing requirements in foreign patent offices, and in part because of the awkwardness resulting from direct literal translations from a foreign language to English. Thus, while the factual determination of the stated utility in an application prepared in the United States may be relatively straightforward,[9] the factual analysis of a foreign priority application to determine the utility disclosed therein may be more laborious and open to varying interpretations.

The weakness of Cross' position is that a fair reading of the pertinent sections of the Japanese priority application as set forth above, discloses utility for the imidazole derivative compounds of the phantom count both as an inhibiting agent for thromboxan synthetase in human or bovine platelet microsomes, as found by the Board, and as therapeutically active agents preventing the biosynthesis of thromboxane A_2, thereby functioning as a medicine preventing deleterious conditions caused by thromboxane A_2, as contended by Cross.

Evidence of any utility is sufficient in the count does not recite any particular utility. Nelson v. Bowler, 626 F.2d 853, 856, 206 USPQ 881, 883 (CCPA 1980). See also Rey-Bellet v. Englehardt, 493 F.2d 1380, 181 USPQ 453 (CCPA 1974); Knapp v. Anderson, 477 F.2d 588, 177 USPQ 688 (CCPA 1973); Blicke v. Treves, 241 F.2d 718, 112 USPQ 472 (CCPA 1957). Here the Board, which is charged with the factual determination of utility,[10] has found that the specification of the Japenese priority application disclosed a utility for the imidazole derivative compounds of the phanton count in the inhibition of thromboxane synthetase in human or bovine platelet microsomes. Inasmuch as the Board is charged with making this factual determination when the issue is raised, inasmuch as they have so done in the instant case, and inasmuch as there is credible evidence to support this factual determination, we are not prepared to say that the Board erred in its

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plant or animal, or it may refer to a particular portion of an organ external to the living organism, e.g., rat aortic loop.

[7] Utility is a fact question. Raytheon Co. v. Roper Corp., 724 F.2d 951, 956, 220 USPQ 592, 596 (Fed. Cir. 1983), cert. denied, 105 S. Ct. 127 (1984). Enablement under §112, paragraph 1, i.e., the how-to-use requirement, is a question of law. Id. at 960 n.6, 220 USPQ at 599 n.6.

[8] While questions one and two are closely connected, a thorough analysis of the utility issue requires first, a determination as to what utility is disclosed, i.e., the stated utility, for the invention claimed in the application. Only after the stated utility has been determined, can a proper analysis be undertaken to determine if the stated utility complies with the "practical utility" requirement of §101. As noted above, these questions regarding utility are factual in nature, see supra note 7, and are to be determined in the first instance by the PTO, the agency with the expertise in this regard.

[9] In applications prepared in the United States by experienced patent drafters, the drafter of the application typically sets forth objectives for the invention in the "Summary of the Invention" section of the application. These objectives will normally be consonant with the utility disclosed for the invention. As this court has noted, "[w]hen a properly claimed invention meets at least one stated objective, utility under §101 is clearly shown." Raytheon Co. v. Roper Corp., 724 F.2d 951, 958, 220 USPQ 592, 598 (Fed. Cir. 1983), cert. denied, 105 S. Ct. 127 (1984).

[10] Under the facts of the instant case, utility and enablement questions are ancillary to priority. In the interference proceeding, Cross raised the issue as to whether the Japanese priority application contained sufficient disclosure to satisfy §112. As noted above, see supra note 5, if Cross prevails on this issue the Japanese priority application would be removed as the basis for awarding priority to Iizuka. See generally 37 C.F.R. §§1.225, .231, .258.

finding as to the stated utility disclosed in the Japanese priority application.

2. Practical Utility

As noted in the preceding part of this opinion, Cross has contended that the Board erred in its finding as to the utility disclosed in the Japanese priority application. This argument may be viewed in a different perspective, we believe, which is that the stated utility in the Japanese priority application, as found by the Board — the inhibition of thromboxane synthetase in human or bovine platelet microsomes[11] — is not sufficiently correlated to a pharmacological activity[12] to be a practical utility. In other words, Cross may be arguing that the minimum acceptable level of utility disclosed in an application claiming a compound having pharmacological activity must be directed to an in vivo utility in order to comply with the practical utility requirement of §101.

The starting point for a practical utility analysis is Brenner v. Manson, 383 U.S. 519, 148 USPQ 689 (1966). The Court in Brenner noted that "a simple, everyday word ["useful," as found in 35 U.S.C. §101] can be pregnant with ambiguity when applied to the facts of life." Id. at 529, 148 USPQ at 693. While noting that "one of the purposes of the patent system is to encourage dissemination of information concerning discoveries and inventions," id. at 533, 148 USPQ at 695, the Court found that a more compelling consideration in the determination of whether a patent should be granted "is the benefit derived by the public from an invention with substantial utility. Unless and until a process is refined and developed to this point — where specific benefit exists in currently available form — there is insufficient justification for permitting an applicant to engross what may prove to be a broad field." Id. at 534-35, 148 USPQ at 695. While we recognize that this case concerned a compound derived from a chemical process, we believe Brenner provides broad guidelines which are helpful in ascertaining what constitutes practical utility for compounds having a pharmacological effect.

In Nelson v. Bowley, 626 F.2d 853, 206 USPQ 881 (1980), our predecessor court, the Court of Customs and Patent Appeals, stated that "[k]nowledge of the pharmacological activity of any compound is obviously beneficial to the public" and concluded that "adequate proof of any such utility constitutes a showing of practical utility." Id. at 856, 206 USPQ at 883.[13] The tests[14] found by the court to be adequate proof of pharmacological activity or practical utility were a rat blood pressure (BP) test and a gerbil colon smooth muscle stimulation (GC-SMS) test. The BP test was an in vivo test, which was deemed by the court to be direct evidence as to the claimed activity, while the GC-SMS test was an in vitro test.[15]

The CCPA in Rey-Bellet v. Englehardt, 493 F.2d 1380, 1383, 181 USPQ 453, 454 (1974), stated that where a count contains no limitation related to utility, evidence establishing a substantial utility for any purpose is sufficient to show a reduction to practice. The court held that three in vivo tests[16] conducted in the United States prior to the filing of Englehardt's U.S. application failed to establish an actual reduction to practice. The court proceeded, however, to find sufficient evidence in the record to establish that Englehardt had conceived a utility for his compound prior to the filing date of his U.S. application. The evidence the court found to be sufficient was testimony by the inventor that he believed his compound would exhibit a particular pharmacological activity because of its structural similarity to another compound which was known to possess the particular pharmacological activity. The court found that the testimonial evidence of Englehardt was corroborated by two exhibits entered into evidence. The evidence adduced by Englehardt was found by the court to be sufficient proof that Englehardt had conceived that his compound had utility for the particular pharmacological activity prior to his U.S. filing date. The court further noted that this was a completed conception of utility because it appeared that nothing beyond the exercise of routine skill would have been required to demonstrate that Englehardt's compound possessed the particular pharmacological utility. While noting that the actual testing done was not sufficient to establish an actual reduction to practice, the court found that the extensive testing done in vivo on animals was routine in nature and was not, therefore, to be construed as an indicator that extensive research, i.e., inventive skill and/or undue experimentation, was required to resolve perplexing intricate difficulties related to the utilization of the compound for the particular pharmacological activity.

The CCPA in Kawai v. Metlesics, 480 F.2d 880, 178 USPQ 158 (1973), concurred with the finding of the Board that the applicants had failed to prove that their foreign priority application was adequate under the patent laws of the United States. The only disclosure in the foreign priority application relating to the compound of the count was that it exhibited "pharmacological effects on the central nervous system," which the applicants conceded was an inadequate disclosure. The applicants, however, relied upon a patent made of record as indicative of the general knowledge of one skilled in the art, which the applicants contended described a compound closely related to their claimed compound, to show utility or pharmacological activity for the compound of the count as an anticonvulsant. The court agreed with the board that there were sufficient structural dissimilarities between the compounds of the patent and those of the count to preclude reliance on the patent to supplement the disclosure deficiencies of the foreign priority application.

In Knapp v. Anderson, 477 F.2d 588, 177 USPQ 688 (CCPA 1973), the court, citing to Blicke v. Treves, 241 F.2d 718, 112 USPQ 472 (CCPA 1957), stated that "[i]t is well settled that if the counts do not specify any particular use, evidence proving substantial utility for any purpose is sufficient to establish an actual reduction to practice." Id. at 590, 177 USPQ at 690 (emphasis added). Noting that the only utility contemplated for the compounds of the count was as ashless dispersants in lubricant compositions used in internal combustion engines, the court found no error in [illegible] that there was no actual reduction to practice because only a potential utility had been established, this holding based upon the Board's finding of a lack of correlation between bench tests and actual service conditions in a combustion engine.

The CCPA has held that nebulous expressions, such as "biological activity" or "biological properties," disclosed in a specification convey little explicit indication regarding the utility of a compound. In re Kirk 376 F.2d 936, 941, 153 USPQ 48, 52 (CCPA 1967). But, while agreeing with the Board that the specification failed to disclose a specific allegation of utility for any compound within the scope of the claims, and that reference in the specification to biological properties [illegible] claimed compound was so general and [illegible] as to be meaningless, the court implied that a disclosure in the specification that the requisite properties of the claimed compounds are similar to those of a natural or synthetic hormone of known activity may, in appropriate circumstances, supplement an application to rectify an inadequate disclosure relating to the practical utility for the compound. Id. at 942, 153 USPQ at 53.

Every utility question arising in an interference, in the final analysis, must be decided on the basis of its own unique factual circumstances. Relevant evidence must be judged as a whole for its persuasiveness in determining whether the suggested use for the compound of the count is a practical utility. Cf. Nelson, 626 F.2d at 858, 206 USPQ at 885.

The Board has found that the Japanese priority application of Iizuka disclosed a practical utility for the compounds of the phantom count in the inhibition of thromboxane synthetase in human or bovine pl[illegible] microsomes, i.e., an in vitro utility. Cle[illegible], this stated utility as found by the Board has been delimited with sufficient specificity to satisfy the threshold requirements of Kawai and Kirk. The stated utility of the Japanese priority application is directed to a specific pharmacological activity possessed by the imidazole derivatives of the phantom count — the inhibition of thromboxane synthetase in vitro. Thus, this court on review is not presented with a general allegation of "biological activity" or "biological properties" as was the CCPA in Kirk, nor is reliance on prior art required to ascertain what specific pharmacological activity the compound of the count possesses, the factual situation confronting the court in Kawai.

The Japanese priority application, moreover, disclosed that it was generally known in the art, as of the critical date, that the parent imidazole and 1-methylimidazole compounds possessed an inhibitory action for thrombox-

[11] A platelet microsome is an in vitro milieu consisting of blood platelets, the small, colorless corpuscles in the blood of all mammals, and other finely granular elements of protoplasm, such as ribosomes, fragmented endoplasmic reticula and mitochondrial christae.

[12] Generally, pharmacological activity refers to the properties and reactions of drugs, especially with relation to their therapeutic value.

[13] For purposes of the present opinion, we consider the phrase "substantial utility," as enunciated in Brenner, to be synonymous with the phrase "practical utility" as used in subsequent opinions of the CCPA.

[14] We recognize that Nelson dealt with tests which were found adequate to establish an actual reduction to practice, as opposed to a constructive reduction to practice. We agree with the Board that principles applicable to a determination of an actual reduction to practice are generally germane to a constructive reduction to practice.

[15] Both parties admitted that the GC-SMS test adequately simulated in vivo smooth muscle stimulation.

[16] The three tests, all in vivo type tests carried out on laboratory animals, were: (1) the Mental Health General Screening Test which indicated the physical response, or absence of a response, of test animals to a drug, indicating the presence, or absence, of a desired pharmacological activity; (2) the Tetrabenazine Antagonism Test which screened drugs for antidepressant activity; and (3) the Sidman Avoidance Test which screened drugs for tranquilizing activity.

ane synthetase. Reliance on this disclosure in the specification of the pharmacological property of the parent imidazole and 1-methylimidazole compounds, as going towards proof of the pharmacological activity of the imidazole derivatives of the phantom count, is particularly relevant in the instant case, we believe, because Iizuka is not relying on this inference to supplement an inadequate disclosure in the Japanese priority application regarding the pharmacological activity of the compound of the phantom count, but rather is relying on this inference as cumulative probative evidence showing an adequately disclosed practical utility in the Japanese priority application.

This court, in Rey-Bellet and Kawai, has implied that a particular pharmacological activity identified with prior art compounds may have probative value as to the fact that the compound of the count possesses this particular pharmacological activity where there is a structural similarity between the prior art compounds and the compound of the count. Rey-Bellet, 493 F.2d at 1385–87, 181 USPQ at 456–58; Kawai, 480 F.2d at 890–91, 178 USPQ at 166–67. Cross has failed to proffer sufficient evidence or present any persuasive arguments going to the question of significant structural dissimilarities between the parent imidazole and 1-methylimidazole compounds and the imidazole derivatives of the phantom count.[11]

The expert of Iizuka, Dr. Ramwell, testified that, as of the critical date, there was an awareness on the part of those skilled in the art that the parent imidazole compound exhibited an inhibitory activity for thromboxane synthetase, in both in vitro and in vivo environments. Dr. Ramwell further testified that there was an awareness by those skilled in the art of a correlation between thromboxane A_2 and platelet aggregation, namely that thromboxane A_2 was a mediator in platelet aggregation. Several exhibits proferred by Iizuka corroborated Dr. Ramwell's testimony as to the general knowledge in the art with respect to the inhibitory effect of the parent imidazole compound for thromboxane synthetase.[12] Accordingly, the similar pharmacological activity of the parent imidazole and 1-methylimidazole compounds have probative value in the factual determination of practical utility for the compounds of the phantom count inasmuch as Cross has not met the burden of proof to establish structural dissimilarities between the parent imidazole and 1-methylimidazole compounds and the imidazole derivatives of the phantom count. Rey-Bellet, 493 F.2d at 1386–87, 181 USPQ at 457.

The Board found that there was adequate proof that the Japanese priority application disclosed a pharmacological activity for the compounds of the phantom count in inhibiting the action of thromboxane synthetase, similar to the pharmacological activity of the parent imidazole and 1-methylimidazole compounds which were found to possess an inhibitory action for thromboxane synthetase, this disclosed knowledge of the inhibitory action of the prior art compounds having been corroborated by testimony and documentary evidence. During the proceedings before the Board, the burden of proof rested upon Cross to show that the Japanese priority application was deficient. 37 C.F.R. §1.257(a). On review, Cross bears the burden of proof to show that the Board erred in finding that the Japanese priority application had adequately disclosed a practical utility. Reviewing the relevant evidence presented to the Board as a whole, we are not persuaded that Cross has met this burden of proof.

[1] The final question we must address is whether the inhibitory activity for thromboxane synthetase in human or bovine platelet microsomes, i.e., an in vitro utility, is sufficient to comply with the practical utility requirement of §101. Based upon the facts of this case, we are not persuaded that the Board erred in finding that the in vitro utility disclosed in the Japanese priority application for the compounds of the count is sufficient to establish a practical utility.

Our predecessor court has noted that adequate proof of any pharmacological activity constitutes a showing of practical utility. See, e.g., Nelson, 626 F.2d at 856, 206 USPQ at 883; Rey-Bellet, 493 F.2d at 1383, 181 USPQ at 454. Dr. Ramwell testified that initial testing of compounds for a particular pharmacological activity is typically done in vitro. In vitro testing permits an investigator to establish the rank order of compounds with respect to the particular pharmacological activity, i.e., to determine the relative potency of the compounds. Compounds having the highest ranking or potency are then selected for further testing in vivo. Presumably this is the accepted practice in the pharmaceutical industry inasmuch as Cross has not proferred any evidence refuting this testimony of Dr. Ramwell, and we note that this practice has an inherent logical persuasiveness. In vitro testing, in general, is relatively less complex, less time consuming, and less expensive than in vivo testing. Moreover, in vitro results with respect to the particular pharmacological activity are generally predictive of in vivo test results, i.e., there is a reasonable correlation therebetween. Were this not so, the testing procedures of the pharmaceutical industry would not be as they are. Iizuka has not urged, and rightly so, that there is an invariable exact correlation between in vitro test results and in vivo test results. Rather, Iizuka's position is that successful in vitro testing for a particular pharmacological activity establishes a significant probability that in vivo testing for this particular pharmacological activity will be successful.

As discussed above, Dr. Ramwell testified that the parent imidazole and 1-methylimidazole compounds had been subjected to both in vitro and in vivo testing as of the critical date, this corroborated by documentary evidence, and found to possess an inhibitory effect for thromboxane synthetase. Based upon this, Dr. Ramwell further testified that he would expect that in vivo testing of the imidazole derivatives of the phantom count would show that these compounds also possessed an inhibitory action for thromboxane synthetase, i.e., there would be a reasonable correlation between in vitro test results and in vivo test results. This evidence was found sufficient by the Board as proof that the Japanese priority application had disclosed a completed practical utility for the imidazole derivative, the phantom count in inhibiting thromboxane synthetase in human or bovine platelet microsomes. Cf. Rey-Bellet, 493 F.2d at 1386–87, 181 USPQ at 457.

[2] Cross argues that the in vitro utility disclosed by the Japanese priority application is not per se useful, and that more sophisticated in vitro tests, using intact cells, or in vivo tests are necessary to establish a practical utility.[13] Cross is arguing that there must be a rigorous correlation of pharmacological activity between the disclosed in vitro utility and an in vivo utility to establish a practical utility. We, however, find ourselves in agreement with the Board that, based upon the relevant evidence as a whole, there is a reasonable correlation between the disclosed in vitro utility and an in vivo activity, and therefore a rigorous correlation is not necessary where the disclosure of pharmacological activity is reasonable based upon the probative evidence. Cf. Nelson, 626 F.2d at 856, 206 USPQ at 883–83.

Our predecessor court has accepted evidence of in vivo utility as sufficient to establish a practical utility. See, e.g., Nelson v. Bowler, 626 F.2d 853, 206 USPQ 881 (CCPA 1980); In re Jolles, 628 F.2d 1322, 206 USPQ 885 (CCPA 1980); Rey-Bellet v. Englehardt, 493 F.2d 1380, 181 USPQ 453 (CCPA 1974).

Opinions of our predecessor court have recognized the fact that pharmacological testing of animals is a screening procedure for testing new drugs for practical utility. See, e.g., In re Jolles, 628 F.2d 1322, 1327, 206 USPQ 885, 890 (CCPA 1980). This in vivo

[11] Contrary to Cross' contention in the Reply Brief, the evidence of record relied upon by Cross to show significant structural dissimilarity appears to us to be directed to the fact that there is a wide disparity in potency for thromboxane synthetase inhibition between the parent imidazole compound and prior art imidazole derivatives. Cross has not directed our attention to any specific evidence of record which establishes, or tends to establish, significant structural dissimilarities between the basic imidazole compound and the imidazole derivatives of the phantom count. Variation in potency, moreover, is a matter of degree of activity, see Bundy, 642 F.2d at 433, 209 USPQ at 51, but is still indicative of activity. There is no requirement that the compounds have the same degree of activity. Id., 209 USPQ at 51. Moreover, this argument may be construed as a tacit admission that the parent imidazole compound does possess the particular pharmacological activity of inhibiting thromboxane synthetase.

Along this line, we note that Dr. Smith, Cross' expert witness, testified generally, based upon the exhibits proffered by Iizuka, see infra note 18, that the parent imidazole compound possessed pharmacological activity for inhibiting thromboxane synthetase, although stating that there was a wide potency spectrum for prior art imidazole derivatives with respect to the parent imidazole compound.

Cross has directed the court's attention to the fact that the Japanese priority application, while disclosing that the parent imidazole and 1-methylimidazole compounds possess an inhibitory action for thromboxane synthetase, further discloses that this inhibitory effect is not satisfactory and that the parent imidazole and 1-methylimidazole compounds have not been put to practical therapeutic use. But a therapeutical utility is not necessarily synonymous to a pharmacological activity. Cf. Nelson, 626 F.2d at 856, 206 USPQ at 883.

[12] For example, Table I in the article "Imidazole: A Selective Inhibitor of Thromboxane Synthetase," PROSTAGLANDINS, Vol. 13, No. 4, April 1977 (Iizuka Exhibit No. 6), lists 1-methylimidazole and the parent imidazole compounds as possessing inhibitory activity for thromboxane synthetase, thereby offering corroboration of Dr. Ramwell's testimony.

The Board noted that Iizuka Exhibits 2-6 and 10-12, while inadmissible for the purpose of establishing the truth of what they say on their face, are admissible to bolster and support the testimony of Dr. Ramwell, as well as for the purpose of establishing what literature was available to the art at the critical time. Thus, for review purposes, we have examined these exhibits for their corroborating value with respect to Dr. Ramwell's testimony.

[13] Cross is seemingly arguing that the in vitro disclosure of the Japanese priority application is only a potential utility. See Knapp v. Anderson, 477 F.2d 588, 591, 177 USPQ 688, 691 (CCPA 1973).

testing is but an intermediate link in a screening chain which may eventually led to the use of the drug as a therapeutic agent in humans. We perceive no insurmountable difficulty, under appropriate circumstances, in finding that the first link in the screening chain, in vitro testing, may establish a practical utility for the compound in question. Successful in vitro testing will marshal resources and direct the expenditure of effort to further in vivo testing of the most potent compounds, thereby providing an immediate benefit to the public, analogous to the benefit provided by the showing of an in vivo utility. Cf. Nelson, 626 F.2d at 856, 206 USPQ at 883.

Today, under the circumstances of the instant case, where the Japanese priority application discloses an in vitro utility, i.e., the inhibition of thromboxane synthetase in human or bovine platelet microsomes, and where the disclosed in vitro utility is supplemented by the similar in vitro and in vivo pharmacological activity of structurally similar compounds, i.e., the parent imidazole and 1-methylimidazole compounds, we agree with the Board that this in vitro utility is sufficient to comply with the practical utility requirement of §101.

3. Enablement

The Board found that the knowledge as to the use of the pharmacological activity disclosed in the Japanese priority application lay in the fact that the system was a microsome system, microsome systems admittedly being known to those skilled in the art. Employing a microsome assay, the skilled worker could determine the relative strength of the compounds of the count vis-a-vis the known parent imidazole and 1-methylimidazole compounds. Thus, the dosage in the microsome assay milieu could be determined without inventive skill or undue experimentation.

Since we have agreed with the Board that the practical utility for the imidazole derivatives of the phantom count lies in their pharmacological activity in the microsome environment, the how-to-use requirement of §112 must be analyzed with reference to the microsome environment. We are confronted with a disclosure, similar to the situation before the court in Bundy, that fails to reveal dosages for the novel compounds per se. 642 F.2d at 434, 209 USPQ at 51. Although the Japanese priority application does disclose the fact that the imidazole derivatives of the phantom count possess a pharmacological activity similar to the parent imidazole and 1-methylimidazole compounds, the priority application, unlike the application in Bundy, does not disclose dosages for the parent imidazole and 1-methylimidazole compounds.

We agree with the Board, however, that this deficiency in the Japanese priority application is not fatal. The testimonial evidence of Dr. Ramwell, corroborated by certain documentary evidence, showed that those skilled in the art had available, at the critical date, information as to approximate dosage levels for the parent imidazole and 1-methylimidazole compounds to produce an IC_{50} effect, i.e., a 50% inhibition of thromboxane synthetase, in a microsome milieu. The objective of the pharmaceutical research undertaken by the parties was to discover imidazole derivatives having a potent inhibitory effect for thromboxane synthetase. Therefore, we believe it is logical, as did the Board, that the starting point for determining IC_{50} dosage levels for the imidazole derivatives of the phantom count would be the IC_{50} dosage levels of the parent imidazole and 1-methylimidazole compounds. The Board found that there was sufficient credible evidence that one skilled in the art, without the exercise of inventive skill or undue experimentation, could determine the IC_{50} dosage level for the imidazole derivatives of the phantom count in the microsome environment. Cf. Bundy, id., 209 USPQ at 51. We do not believe the Board erred in arriving at this conclusion. This is not a case such as In re Gardner, 427 F.2d 786, 166 USPQ 138 (1970), where the CCPA held that the applicant's disclosure was nonenabling because inventive skill and undue experimentation would be required to discover approprite dosages for humans, i.e., a therapeutic use. In the instant case, we are confronted with a pharmacological activity or practical utility, not a therapeutic use.

While we agree with the Board that the disclosure in the Japanese priority application is somewhat confusing with respect to the 2.5×10^{-8} level of molar concentrations, and that the 2-[p-(1-imidazolylmethyl) phenoxy]-acetic acid hydrochloride compound is outside the phantom count of the interference, this disclosed molar concentration, we believe, does provide some probative value going towards the sufficiency of the Japanese priority application for an enabling disclosure. The disclosed molar concentration would provide sufficient information as to an initial dosage level so that one skilled in the art could determine, without inventive skill or undue experimentation, the necessary molar concentrations for the imidazole derivatives of the phantom count to achieve the desired pharmacological effect, i.e., the 50% inhibition of thromboxane synthetase in human or bovine platelet microsomes.

[3] The Board held the disclosure of the Japanese priority application adequate to satisfy the first paragraph of §112. The burden is on Cross to show Board error in arriving at this conclusion, and we are not persuaded that Cross has successfully carried this burden. Accordingly, we are satisfied that the how-to-use requirement of §112 has been complied with by the disclosures of the Japanese priority application.

Affirmed.

Court of Appeals, Federal Circuit

In re National Data Corporation

No. 84-1137

Decided Jan. 30, 1985

TRADEMARKS

1. Identity and similarity — How determined — Descriptive or disclaimed matter (§67.4061)

Technicality of disclaimer in application to register mark has no legal effect on issue of likelihood of confusion, public being unaware of what words have been disclaimed during prosecution of application, nor can fact that applicant voluntarily disclaimed words as tactical strategy, believing that it would assist in avoiding holding of likelihood of confusion with another's mark, affect scope of protection to which another's mark is entitled.

2. Identity and similarity — How determined — Descriptive or disclaimed matter (§67.4061)

Applicant was entitled to show that component of registered mark was descriptive and its proofs should not have been disregarded on ground that registration could not be attacked, since registration affords prima facie rights in mark as whole, not in any component, so that showing of descriptiveness or genericness of part of mark does not constitute attack on registration.

3. Identity and similarity — Words — Similar (§67.4117)

"Cash Management Account" and "The Cash Management Exchange" are, in large part, identical in sound and appearance, have general similarity in cadence, and, while not synonyms, both connote monetary transactions, sole differing feature being insufficiently different to distinguish marks to public.

Appeal from Patent and Trademark Office Trademark Trial and Appeal Board; 222 USPQ 515.

Application for registration of service mark of National Data Corporation, Serial No. 294,193. From decision affirming refusal to register, applicant appeals. Affirmed.

Stephen A. Bent, and Schwartz, Jeffrey, Schwabb, Mack, Blumenthal & Koch, P.C., both of Alexandria, Va. (Peter G. Mack, Alexandria, Va., on the brief) for appellant.

Thomas E. Lynch, Associate Solicitor (Joseph F. Nakamura, Solicitor, and Jere W. Sears, Deputy Solicitor, on the brief) for appellee.

Before Davis, Smith, and Nies, Circuit Judges.

Nies, Circuit Judge.

I.

Background

National Data Corporation filed an application to register THE CASH MANAGEMENT EXCHANGE on the Principal Register as a service mark for "computerized ... management services." Use of the mark is alleged since on or about November 18, 1980. The examiner refused registration under §2(d) of the Trademark Act of 1946, as amended, 35 U.S.C. §1052(d) (1976), on the ground that the mark sought to be registered so resembled the following mark as to be likely to cause confusion, or to cause mistake or to deceive:

CASH MANAGEMENT ACCOUNT
Reg. No. 1,118,929, issued May 22, 1979
for "financial services involving the use of plastic credit cards by the card holders for loans to card holders from their brokerage equity account."

No disclaimer of rights in CASH MANAGEMENT appears in the registration for CASH MANAGEMENT ACCOUNT.

A second basis for rejection was given under §2(e), 35 U.S.C. §1052(e) (1976), on the ground that the words CASH MANAGEMENT, as well as the word EXCHANGE

Date: October 1992

1880

Recombinant Adenovirus is an Efficient Vector for *In Vivo* Gene Transfer and Can be Preferentially Directed at Vascular Endothelium or Smooth Muscle Cells

John E. Willard, Michael E. Jessen, Robert D. Gerard, and Robert S. Meidell. U of Texas Southwestern Medical Center, Dallas, TX

Previous attempts to genetically modify vascular endothelial and smooth muscle cells *in vivo* have used liposome mediated transduction, direct DNA injection, or recombinant retroviral vectors. Since gene transfer by these methods is inefficient, they are unlikely to alter biologic properties of large numbers of cells. Recombinant adenoviruses have several characteristics which make them attractive vectors for foreign gene transfer: (a) viral stocks with titers of $\geq 10^{10}$ pfu/ml can be readily obtained; (b) adenoviruses promiscuously infect a wide range of mammalian species and cell types; (c) available vectors will accept foreign genes up to 7kb; (d) in the absence of AdE1A, adenoviral genes are not expressed; (e) rapid infection kinetics permit brief exposure of the target cell population; and (f) gene transduction and expression are independent of target cell division. This study was performed to assess the efficacy of *in vivo* adenoviral gene transfer and expression in rabbit vascular endothelial and smooth muscle cells. A recombinant adenovirus containing a gene encoding nuclear-localized ß-galactosidase expressed from the cytomegalovirus promoter (AdCMV-nLac) was delivered by (a) direct injection into jugular veins isolated by proximal and distal ligatures and allowed to dwell for 30 minutes or (b) perforated balloon catheter infusions into the wall of carotid arteries. Vessel segments were harvested at 4 days, fixed, stained with X-gal and eosin, and sectioned. Histologic analysis of vein segments revealed highly efficient (20-30%) expression of ß-galactosidase limited to the endothelium, whereas expression in arterial wall was less efficient and limited to the site of medial disruption. Thus, adenovirus is an efficient vector for *in vivo* gene transfer to vascular tissue and it can be preferentially directed at specific layers of the vessel wall.

Arteriosclerosis: Biology of the Vessel Wall
Wednesday Morning

1881

Nitric Oxide Synthase is Expressed by Endothelial Cells Overlying Human Atherosclerotic Plaques.

Cynthia L. Sundell, Philip A. Marsden, Romesh R. Subramanian, Jennifer S. Pollock, David G. Harrison and Josiah N. Wilcox, Department of Medicine, Emory University, Atlanta, GA

Atherosclerosis is associated with reduced endothelial-derived relaxing factor (EDRF) activity. To determine whether this is due to decreased synthesis of nitric oxide (NO) synthase, studies were conducted on normal baboon tissues and normal and atherosclerotic human vessels by in situ hybridization (ISH) and immunocytochemistry (ICC) with probes specific for the constitutive calcium-regulated endothelial NO synthase. NO synthase mRNA was detected by ISH in a subset of endothelial cells in all normal baboon tissues examined (cerebellum, kidney, spleen, adrenal gland and small intestine). NO synthase mRNA and protein were also detected in luminal endothelial cells and subsets of endothelial cells in the adventitial vessels of normal baboon and human aorta. In order to determine whether NO synthase expression may be altered in atherosclerosis, human aortic fatty streaks and carotid endarterectomy specimens were studied. NO synthase mRNA and protein were found normally expressed in the luminal and adventitial endothelial cells of human aortic fatty streaks. NO synthase expression was also detected in endothelial cells overlying fibrous caps of old carotid atherosclerotic plaques containing well-developed necrotic cores. These data suggest that the loss of EDRF activity associated with atherosclerosis is not due to an alteration of endothelial NO synthase expression.

1882

Inhibition of Macrophage Nitric xide Synthase by Oxidized LDL

Xiaochun Yang, Robert R. Sciacca, Paul J. Cannon, Columbia University, New York, NY

Macrophages activated by cytokines synthesize nitric oxide (NO) which is vasodilator and cytotoxic. To investigate the effects of low density lipoproteins (LDL) on NO synthesis, J744 macrophages were incubated with native LDL (n-LDL), copper oxidized LDL (ox-LDL) and acetylated LDL (ac-LDL) for 24 hours and were activated with 100U IFN-γ and 5 ug/ml LPS. NO synthase (NOS) activity was assessed from nitrite accumulation in the media and by the capacity of a 100,000 x g. supernatant of cell homogenate to form nitrite and citrulline from l-arginine. Incubation with ox-LDL (25 μg of protein/ml) resulted in significantly decreased NO production (45 ± 15 nmoles/ml) in comparison to control LPDS (79 ± 16) and n-LDL (85 ± 19), $p < .01$. Ac-LDL did not significantly inhibit NOS. The effect of ox-LDL was dose-dependent and exhibited non-competitive kinetics (substrate range of 40μM - 200μM arginine in cell supernatant) with an IC_{50} of 25 μg of protein/ml. Inhibition of NO synthase was also produced by ox-LDL lipids extracted from ox-LDL and by phosphytidyl choline (PC) vesicles containing lysophosphytidyl choline, whereas n-LDL, lipid extracted from n-LDL and PC vesicles did not inhibit the enzyme. The data indicate that ox-LDL inhibits nitric oxide synthase in activated macrophages. Impaired NO synthesis by "foam" cells containing ox-LDL may contribute to impaired vasodilator responses of atherosclerotic blood vessels.

1883

"Nitric Oxide and Monocyte Chemotaxis"

Sergei N. Belenky, Richard A. Robbins, Israel Rubinstein, University of Nebraska Medical Center, Omaha, Nebraska.

The role of nitric oxide (NO) in vascular disease is unclear. In order to clarify the role of NO in the chemotaxis of monocytes, normal peripheral blood human monocytes were purified and their chemotactic activity evaluated in response to formyl-methyl-leucyl-phenylalanine. Three inhibitors of nitric oxide synthase N^G-monomethyl-L-arginine (L-NMMA), N^G-nitro-L-arginine-methyl-ester (L-NAME), and L-canavanine were evaluated for their capacity to inhibit monocyte chemotaxis. Each resulted in a significant reduction of monocyte chemotactic activity ($p<0.01$). The enantiomeric specificity of one inhibitor, L-NMMA, was evaluated by evaluating D-NMMA. D-NMMA caused no reduction in monocyte chemotactic activity. Because NO is generated from L-arginine and proposed to exert its effects by upregulating guanyl cyclase, thus increasing intracellular levels of cGMP, the capacity of L-arginine or cGMP to reverse the inhibition of monocyte chemotaxis was evaluated. Both L-arginine and cGMP caused a dose dependent reversal of L-NMMA inhibition of monocyte chemotaxis. The above data suggest a role for nitric oxide (NO) in the migration of monocytes and may have important implications in the generation of atherosclerotic plaques.